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Research Notes: Soybean tissue culture

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1) Soybean tissue culture.

The recently developed techniques of plant tissue culture have been used to develop somatic cell genetics programs in solanaceous species, particularly *Petunia, Nicotiana,* and *Datura* (Vasil et al., 1979). Development of tissue culture techniques has permitted the isolation of biochemical mutants, production of somatic hybrids, and recovery of haploid plants. Unfortunately, extrapolation of these techniques to leguminous and graminaceous species of greater economic importance has proven quite difficult. Recent reports of plant regeneration from protoplasts of alfalfa, a legume (Kao and Michayluk, 1980) and pearl millet, a cereal (Vasil and Vasil, 1980), suggest that development of tissue culture techniques may be feasible for the more recalcitrant legumes such as soybean. This note is a summary of recent research in our laboratory with soybean tissue culture.

A. Plant regeneration Explants for plant regeneration experiments were derived from the albino soybean, y_{11}/y_{11}, and from the Canadian soybean cv. 'Maple Arrow'. All attempts to regenerate plants from mature leaf or stem segments of soybean have been unsuccessful. However, multiple shoot formation was induced from cultured cotyledon axillary bud explants. Axillary buds were excised from sterile 6-14-day-old seedlings from seeds sown on 10 g/l agar. Single bud explants placed on either Medium 1 or 2 (Table 1) produced a mean of 5 shoots per explant within 4 wks. Shoots could be separated and transferred to Medium 3, the soybean high rooting medium (HRM) published earlier (Evans et al., 1976), and each shoot transferred to HRM produced roots within 2 wks. Rooted plantlets of Maple Arrow then were transferred to the greenhouse where each plant grew to maturity. Chromosomes were counted in root tips of five regenerated plants and each contained 2n=40 chromosomes. This method of plant regeneration does not permit large scale plant propagation from mature explants, but will permit production of up to 10 plants from explants derived from a single seedling.

B. Cell suspension cultures Callus was initiated from hypocotyl sections of 9-day-old seedlings cultured on Medium 4. After 4 wks a friable white callus was obtained. Callus was transferred to a 60 X 15 mm disposable plastic Petri dish containing 3 ml of liquid culture Medium 5, and shaken at

Table 1

Culture media used for soybean experiments

Medium	Value
MS + 740 µM Ade 1 µM 6BA 0.1 µM NAA	multiple shoot formation from dormant cotyledon
MS + 740 µM Ade 1 µM 6BA 0.1 µM IBA	axillary buds
MS + 11 µM NAA 2 µM KIN 16 µM NicoA	root formation
MS + 1 µM KIN 1 µM IBA	callus formation for initiation of stable suspension culture
MS + 1 µM 2,4-D	maintenance of suspension cultures

MS = Murashige and Skoog (1962) macro and micronutrients with Gamborg B5 vitamins (Gamborg, 1975).

50 rpm. Three ml of fresh liquid Medium 5 were added 7 days after culture initiation. Four days later, the culture was transferred to a 250 ml flask with 20 ml of Medium 5. Afterwards cells were subcultured into new Medium 5 every 4 days. Cells from suspension cultures were prepared for chromosome counts as described (Evans and Reed, 1980), using cells 24 hrs after subculture. Cell suspension cultures with stable chromosome number, 2n = 40, were produced and maintained from both y_{11}/y_{11} albino soybean and Maple Arrow.

C. Plant protoplasts Protoplasts were readily isolated from the soybean suspension cultures 3 days after each subculture. Two ml of suspension culture were mixed with 2 ml of a protoplast isolation solution containing 2% Onozuka R10 (Kinki Yakult), 1% pectinase (Sigma), and 1% hemicellulase (Rohm and Haas) dissolved in 0.7 M glucose, 3 mM MES buffer, 6 mM $CaCl_2$, and 0.7 mM NaH_2PO_4 at pH 5.5. This mixture was incubated in the dark at room temperature and shaken at 50 rpm. Protoplasts were released in 6-8 hrs. Cellular debris was removed by filtration through a 44 µm filter, followed by centrifugation

twice at 100 g. Protoplasts were resuspended, then cultured in Medium 8p of Kao and Michayluk (1975). The protoplasts reformed cell walls within 24 hrs of transfer to culture medium and divided by day 2. A large fluffy white callus was produced from each soybean protoplast culture, but plant regeneration from this callus has not occurred to date.

Development of a cellular genetics system with soybean is limited by the present inability to regenerate intact plants from single cells. The work reported here suggests that most other techniques useful for plant somatic cell genetics, i.e., callus culture, cell suspension culture, and protoplast isolation and culture are similar to previously reported species and can be exploited without difficulty. Hopefully more efficient methods of plant regeneration will be achieved in the near future.

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